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SODIUM SACCHARIN

TOXICITY and TERATOGENICITY STUDIES in Avian Embryos

FDA Contract #71-330

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STUDIES on the TOXICITY and TERATOGENICITY of SODIUM SACCHARIN

SUMMARY and CONCLUSIONS

Sodium saccharin was solublized in 10% ethanol for air cell administration and in water for use in the yolk injection protocols. The maximum dose levels employed in these studies was 200 mg/kg (10 mg/egg).

Mortality data for the air cell-96 hr series suggest that sodium saccharin was toxic to chicken embryos at 80 mg/kg and above. Estimates of LD-50 in this series were 69.4 mg sodium saccharin/kg with a range of 47.3 - 105.9. The mortality data for the other three test protocols were not significantly different by chi-square analyses in comparison with solvent controls.

Teratological findings indicated that sodium saccharin produced a high incidence of hypopigmentation of the down in each of the four test protocols. Hypopigmentation was classified as a toxic response and was not found to persist in those chicks maintained to six months of age and fed a normal diet. Xanthophyll deposition was normal in the chicks within two weeks after hatching.

Head, limb, skeletal, and visceral abnormalities resulting from sodium saccharin administration were not statistically significant for any of the individual dose levels in any of the four test protocols; however, when all doses of the air cell-0 hr series were combined for comparison with the solvent control groups, a chi-square value of 22.08 (5 DF) was obtained. This value was significant above the 0.005 level of probability and strongly suggests that sodium saccharin may be teratogenic. Post hatch data failed to reveal a carryover effect from sodium saccharin administration during incubation.

GENERAL PROCEDURES

The protocols as specified under FDA Contract #71-330 were followed in the investigation of toxicity and potential teratogenicity of the specified substance. The toxicity of the substance was evaluated from the percentage hatch of embryos injected either in the air cell or yolk at either zero hours (post-incubation) or after 96 hours incubation to provide four separate evaluations.

EGG SOURCE AND HANDLING

All eggs used in these investigations were from Shaver Starcross pullets housed at the Poultry Research Center of the University of Arizona in Tucson. The parent stock was maintained on the University of Arizona breeder diet which had been formulated to provide more than adequate amounts of all the known nutrients required by the breeding hen.

The feed was specially prepared to assure no contaminations and did not contain any additive drugs such as antibiotics. All eggs prior to use (within 48 hours of lay) were candled to remove any containing blood spots, abnormal air cells or abnormal shells, and only clean eggs ranging in weight from 23 - 26 ounces per dozen were used.

The supply flock was tested to assure the absence of Pullorum and Mycoplasma gallisepticum.

The eggs were incubated in forced draft Jamesway 252 machines with automatic temperature and humidity controls and an automatic turning device.

COMPOUND HANDLING FOR INJECTION

The substance tested was solubilized in a number of the prescribed solvents in order to determine the maximum concentrations which could be employed. Where possible, water was the solvent of choice. Maximum

injection volume was 0.05 ml. and all solvents and glassware were autoclaved prior to preparation of the solutions for use. The dose levels were administered with a microliter syringe using sterilized needles.

The preliminary range-finding studies using each of the administration routes and times were carried out with 10 - 25 eggs per dose level and included solvent controls, untreated controls and either drilled or pierced controls.

The actual dose-response protocol was carried out in two or more injections on different days to produce a minimum of 100 eggs at each dose level in five or more levels selected from the range- finding studies.

EXAMINATIONS OF EMBRYOS AND CHICKS

Eggs were candled daily and the dead embryos removed, examined and any abnormalities recorded. Five chicks from each dose level in each hatch were X-rayed to determine any skeletal abnormalities. Additional eggs injected at the approximate LD-50 level and an additional level below that were incubated and embryos at 8, 14, 17 days and hatch chicks removed for histopathological examinations.

In additional studies representative chicks from the dose-response protocol were saved. These chicks were housed in electrically-heated battery brooders with raised wire floors and fed University of Arizona diets. Feed consumption and growth rates were evaluated at 6 weeks of age and a sample of the birds sacrificed for gross and histopathological examinations.

The remaining birds in each group were maintained to 6 months of age and then sacrificed.

DATA HANDLING

All data were coded on forms provided by FDA for computer input. In addition to summaries of mortalities and abnormalities, a number of statistical evaluations were carried out. These statistical analyses included the following for both mortality and the incidence of abnormal embryos:

1. Chi-square tests for all dose levels and for each level against the solvent control.
2. Linear regression analyses + chi square test of linearity.
   a. % response against dose
   b. % response against log dose
   c. log % response against dose
   d. arcsin transformation against dose
   e. arcsin transformation against log dose
3. Log dose against Probit using Finney's maximum likelihood method.
   a. Where significant, the LD-30, 50, 70 and 90's were estimated with 95% confidence intervals.
4. One-way analyses of variance.
5. Linear regression with replication.

Sodium saccharin (71-11) was solublized in 10% ethanol for use in the protocols requiring air cell injection and in water for yolk administration. The highest dose level employed was 200 mg/kg (10 mg/egg).

MORTALITY

The mortality data obtained in the four test protocols are shown in Tables 1 - 4. Chi-square analyses of these data (Table 5) indicated that only air cell administration in 96-hr embryos increased embryo mortality. Dose levels of 80 mg/kg administered in 10% ethanol resulted in significant ($P < 0.05$) increases in mortality rate. Probit analyses of these data yielded an LD-50 estimate of 69.4 mg/kg with a range of 47.3 - 105.9 (Table 6). These results indicated that sodium saccharin was embryo toxic to the developing chick embryo. Dose levels of 80 or 160 mg/kg resulted in 73 - 75% embryo mortalities in comparison with a 34.6% incidence obtained with the injection of the 10% ethanol solvent.

TERATOLOGY

The data obtained for the occurrence of abnormal embryos and those showing head, skeletal, visceral, and limb abnormalities are shown in Tables 1 - 4 for the four test protocols. Employing air cell administration at 0 hrs, 30.4 and 27.3% of the embryos injected with 80 or 160 mg/kg, respectively, were abnormal. The majority of these abnormalities were classed as toxic responses and exhibited hypopigmentation (bleached) (Table 1). One of the embryos injected with the 10% ethanol solvent exhibited beak agenesis and none of these had hypopigmentation (Table 10). Chi-square analyses of the air cell-0 hr abnormality data are shown in Table 7. The two higher dose levels (80 & 160 mg/kg) showed significantly higher abnormality incidences ($P < 0.05$). A comparison of all dose levels in

this protocol, ranging from 10 - 160 mg/kg with the groups receiving the 10% ethanol solvent resulted in a chi-square value of 102.14 with 5 degrees of freedom which was significant above the 0.005 level of probability.

Air cell-96 hr administration of sodium saccharin at dose levels of 80 mg/kg and above also resulted in significant increases by chi-square analyses in the incidence of abnormalities. These dose levels also resulted in an 8 - 14 incidence of hypopigmentation (Tables 2 & 7).

Yolk-0 hr administration of sodium saccharin in water also resulted in hypopigmentation at dose levels of 10 mg/kg and above. Each of the dose levels employed in this protocol resulted in statistically significant chi-square values for abnormality incidence in comparison with solvent controls (Table 7).

Yolk-96 hr administration of sodium produced a statistically significant increase in the occurrence of abnormal embryos (Tables 4 & 7).

Linear regression analyses of log dose against probit of abnormality incidence were not statistically significant (Table 8).

Data on H-S-V-L abnormalities for the four test protocols are shown in Tables 1 - 4. None of the individual dose levels in the four test protocols resulted in a statistically significant chi-square value for H-S-V-L abnormalities in comparison with solvent controls (Table 9). Air cell-0 hr administration of sodium saccharin did significantly increase the incidence of H-S-V-L abnormalities when all dose levels were combined for comparison with the 10% ethanol solvent injections. This comparison yielded a chi-square value of 22.08 with 5 degrees of freedom and was statistically significant above the 0.005 level of probability (Table 9). These data suggest that sodium saccharin may be

[redacted] teratology findings are

POST HATCH DATA

Chicks which had received 10 - 160 mg sodium saccharin/kg were maintained to six months of age (Table II). No apparent differences were noted in either body weights at one day, six weeks or six months which could be related to sodium saccharin administration. Feed consumption was normal for all groups and sexual maturity was essentially comparable regardless of prior treatment.

The hypopigmentation of the down which was apparent at hatching did not adversely affect normal skin pigmentation resulting from xanthophyll deposition. Feather development was normal in all chicks.

Table 1

Sodium Saccharin

in 10% Ethanol

Air Cell - 0 hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
160.0	143	39.16	56	27.27	39	6.99	10	2.79	4			4.19	6			0.69	1	19.58	28		
80.0	102	22.54	23	30.39	31	1.96	2	0.98	1			0.98	1					29.41	30		
40.0	102	26.47	27	0.98	1	0.00	0									0.98	1				
20.0	103	26.21	27	0.00	0	0.00	0														
10.0	100	28.00	28	3.00	3	1.00	1	1.00	1							1.00	1			1.00	1
0.0	62	29.03	18	1.61	1	1.61	1	1.61	1												
drilled	140	12.85	18	1.42	2	1.42	2	0.71	1			0.71	1								
untreated	506	12.25	62	1.97	10	1.38	7	0.39	2			0.59	3	0.39	2	0.19	1	0.39	2		

SUMMARY - ALL DOSE LEVELS

550	29.27	161	13.45	74	2.36	13	1.09	6			1.27	7			0.55	3	10.55	58	0.18	1

Table 2

Sodium Saccharin

in 10% Ethanol

Air Cell - 96 hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
160.0	105	73.33	77	17.14	18	0.95	1	0.95	1							1.90	2	14.28	15		
80.0	148	75.00	111	13.51	20	4.72	7	2.02	3			2.70	4			1.35	2	8.10	12		
60.0	51	21.56	11	5.88	3	5.88	3					5.88	3								
40.0	101	29.70	30	0.00	0	0.00	0														
20.0	100	17.00	17	5.00	5	3.00	3	1.00	1			1.00	1	1.00	1	1.00	1	1.00	1		
10.0	101	13.86	14	0.99	1	0.99	1	0.99	1												
0.0	52	34.61	18	1.92	1	3.84	2	1.92	1					1.92	1						
drilled	247	19.02	47	1.21	3	0.80	2	0.80	2							0.40	1				
untreated	506	12.25	62	1.97	10	1.38	7	0.39	2			0.59	3	0.39	2	0.19	1	0.39	2		

SUMMARY - ALL DOSE LEVELS

606	42.90	260	7.76	47	2.48	15	0.99	6		1.32	8	0.17	1	0.83	5	4.62	28		

Table 3

Sodium Saccharin

In Water

Yolk-0 hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
200.0	129	42.63	55	34.10	44	0.77	1							0.77	1			34.10	44	0.77	1
160.0	130	36.92	48	44.61	58	0.76	1			0.76	1							43.84	57		
80.0	100	46.00	46	51.00	51	0.00	0											51.00	51		
40.0	130	47.69	62	34.61	45	0.00	0									0.76	1	33.84	44		
10.0	129	46.51	60	9.30	12	2.32	3	0.77	1	0.77	1	0.77	1			1.55	2	6.97	9		
0.0	196	47.44	93	1.02	2	1.02	2					1.02	2			1.02	2				
pierced	70	42.85	30	1.42	1	1.42	1					1.42	1								
untreated	506	12.25	62	1.97	10	1.38	7	0.39	2			0.59	3	0.39	2	0.19	1	0.39	2		

SUMMARY - ALL DOSE LEVELS

618	43.85	271	32.36	200	0.81	5	0.16	1	0.32	2	0.16	1	0.16	1	0.49	3	33.17	205	0.16	1

Table 4

Sodium Saccharin

in Water

Yolk - 96 hrs

Dose, ppm	No. Fertile	Mortality		Abnormal				Abnormalities by category											
				Total		H-S-V-L		Head		Skeletal		Viscera		Limbs		Structural		Toxic Response		Functional	
		%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#
200.0	50	16.00	8	4.00	2	0.00	0									4.00	2				
160.0	100	17.00	17	1.00	1	1.00	1							1.00	1			1.00	1	1.00	1
80.0	102	11.76	12	4.90	5	0.00	0											4.90	5		
40.0	102	11.76	12	6.86	7	0.00	0											6.86	7		
20.0	99	20.20	20	2.02	2	2.02	2	1.01	1					1.01	1						
10.0	52	7.69	4	9.61	5	0.00	0											9.61	5		
0.0	151	15.89	24	0.00	0	0.00	0														
pierced	49	4.08	2	2.04	1	2.04	1	2.04	1												
untreated	506	12.25	62	1.97	10	1.38	7	0.39	2			0.59	3	0.39	2	0.19	1	0.39	2		

SUMMARY - ALL DOSE LEVELS

505	14.46	73	4.36	22	0.59	3	0.20	1				0.40	2	0.40	2	3.56	18	0.20	1

Table 5

Sodium Saccharin

Chi-Square Analyses of Mortality

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
10.0	0.00	7.73*(less)	0.00	1.55
20.0	0.05	5.04*(less)	——	0.50
40.0	0.03	0.19	0.01	0.55
60.0	——	1.57	——	——
80.0	0.55	25.67*	0.01	0.55
160.0	1.51	20.23*	3.11	0.00
200.0	——	——	0.55	0.04
All Doses (DF)	9.92 (5)	182.83*(6)	4.71 (5)	6.45 (6)

* Probability < 0.05-0.005.

Table 6

Sodium Saccharin

Probit Analyses - Mortality

	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
LD-30 (Range)	NS	37.5 (17.8-53.8)	NS	NS
LD-50 (Range)	NS	69.4 (47.3-105.9)	NS	NS
LD-70 (Range)	NS	128.4 (88.1-298.1)	NS	NS
LD-90 (Range)	NS	312.1 (171.7-1674.3)	NS	NS

Table 7

Sodium Saccharin

Chi-Square Analyses of Abnormalities

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
10.0	0.00	0.07	11.01*	11.15*
20.0	0.07	0.24	———	1.06
40.0	0.14	0.12	68.80*	8.26*
60.0	———	0.28	———	———
80.0	18.55*	4.34*	109.15*	5.23*
160.0	16.54*	6.21*	96.03*	0.04
200.0	———	———	67.40*	2.72
All Doses (DF)	102.14*(5)	40.55*(6)	147.42*(5)	18.48*(6)

* Probability < 0.05-0.005.

Table 8

Sodium Saccharin

Probit Analyses - Abnormalities

Air Cell		Yolk	
0 hrs	96 hrs	0 hrs	96 hrs
NS	NS	NS	NS

Table 9

Sodium Saccharin

Chi-Square Analyses of HLSV Abnormalities

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
10.0	0.15	0.07	0.13	0.00
20.0	0.07	0.02	———	1.06
40.0	0.06	0.12	0.19	0.00
60.0	———	0.28	———	———
80.0	0.14	0.23	0.07	0.00
160.0	1.52	0.06	0.13	0.04
200.0	———	———	0.13	———
All Doses (DF)	22.00* (5)	10.42 (6)	2.11 (5)	7.22 (5)

* Probability < 0.05-0.005.

TABLE 10
SODIUM SACCHARIN

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Untreated Control	506	10	1	fatty metamorphosis-liver
			2	malposition
			1	anophthalmia-unilateral; agenesis-maxilla
			1	dysalliognathia-beak; dysgnathia-beak
			1	celosomia-abdomen
			1	dwarfism
			1	ankylosis-hindlimb, unilateral
			1	fusion failure-abdomen
			1	agenesis-toe, unilateral
Drilled Control - 0 hrs	140	2	1	celosomia-abdomen
			1	abnormal shortening-maxilla and beak
Drilled Control - 96 hrs	247	3	1	anophthalmia-unilateral
			1	dwarfism
			1	anophthalmia-bilateral
Pierced Control - 0 hrs	70	1	1	celosomia-abdomen
Pierced Control - 96 hrs	49	1	1	exencephaly; aplasia-down
In Water Yolk - 0 hrs 200.0 mg/kg	129	44	43	complete hypopigmentation-down
			1	fusion failure - yolk sac; ankylosis-hindlimb, unilateral; complete hypopigmentation-down
160.0 mg/kg	130	58	51	complete hypopigmentation-down
			6	slight hypopigmentation-down
			1	abnormal curvature-vertebral column

TABLE 10
SODIUM SACCHARIN

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In Water- Yolk 0 hrs 80.0 mg/kg	100	51	51	slight hypopigmentation-down
40.0	130	45	1	dwarfism
			28	complete hypopigmentation-down
			16	slight hypopigmentation-down
10.0	129	12	9	slight hypopigmentation-down
			2	dwarfism
			1	anophthalmia-unilateral; abnormal shortening-maxilla; musculoskeletal malformation-spinal cord; celosomia-abdomen
0.0	196	2	2	dwarfism; celosomia-abdomen
In Water - Yolk 96 hrs 200.0	50	2	2	dwarfism
160.0	100	1	1	ataxia; malrotation-hindlimb, unilateral; complete hypopigmentation-down
80.0	102	5	5	complete hypopigmentation-down
40.0	102	7	7	slight hypopigmentation-down
20.0	99	2	1	edema-ankle, unilateral
			1	agenesis-eyelid, unilateral
10.0	52	5	5	complete hypopigmentation-down
0.0	151	0	0	

TABLE 10
SODIUM SACCHARIN

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In 10% Ethanol Air Cell - 0 hrs 160.0 mg/kg	143	59	1	dwarfism
			1	acrania; agenesis-maxilla
			1	anophthalmia-bilateral
			2	degeneration-renal tubule
			1	astrocytoma-brain
			4	granulation tissue-renal tubule
			1	malacia-brain
			28	complete hypopigmentation-down
80.0	102	31	1	malacia-brain; fatty metamorphosis-liver
			30	slight hypopigmentation-down
40.0	102	1	1	dwarfism
20.0	103	0	0	
10.0	100	3	1	dwarfism
			1	fusion failure - yolk sac
			1	exophthalmia-unilateral; dysgnathia-beak
0.0	62	1	1	agenesis-beak

TABLE 10
SODIUM SACCHARIN

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In 10% Ethanol Air Cell - 96 hrs 160.0 mg/kg	105	18	15	complete hypopigmentation-down
			1	cyclopia; agenesis-maxilla
			2	dwarfism
80.0	148	20	12	complete hypopigmentation-down
			1	anophthalmia-unilateral; dwarfism
			1	dwarfism
			1	anophthalmia-unilateral; dysgnathia-beak
			1	anophthalmia-bilateral
			1	vacuolization-renal tubule
			2	granulation tissue-renal tubule
			1	degeneration-renal tubule
60.0	51	3	3	granulation tissue-renal tubule
40.0	101	0	0	
20.0	100	5	1	fatty metamorphosis-liver
			1	encephalocele
			1	dwarfism
			1	contracture-toe, unilateral
			1	hemorrhage-kidney

TABLE 10
SODIUM SACCHARIN

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In 10% Ethanol Air Cell - 96 hrs 10.0 mg/kg	101	1	1	dysgnathia-beak
0.0	52	1	1	agenesis-head; ectromelia-wing, bilateral; ectrosyndactyly-toe, bilateral

TABLE 11

SODIUM SACCHARIN

POST HATCH DATA

Injection Date - 9/7/71

Label	Dose mg/kg	Age at Sexual Maturity Days	Body Weight at Hatch gm	Body Weight 6 wks gm M	Body Weight 6 wks gm F	Body Weight 6 mo. kg M	Body Weight 6 mo. kg F	Feed Consumption 6 wks, gm	Feed Consumption 6 mo. kg
201	10.0	140	40.9	490	394	1.76	1.48	1085	12.6
202	20.0	141	41.9	487	406	1.76	1.48	1036	12.9
203	40.0	139	42.9	428	398	1.78	1.74	838	12.8
204	80.0	137	40.9	470	382	2.04	1.63	897	13.4
205	160.0	145	40.4	496	409	1.97	1.89	919	13.1
Control	-	146	40.4	448	411	204	1.67	843	13.0